

# Cellular Response to Bleomycin-induced DNA Damage in Human Fibroblast Cells in Space



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## Introduction

Outside the protection of the geomagnetic field, astronauts and other living organisms are constantly exposed to space radiation that consists of energetic protons and other heavier charged particles. Whether spaceflight factors, microgravity in particular, have effects on cellular responses to DNA damage induced by exposure to radiation or cytotoxic chemicals is still unknown, as is their impact on the radiation risks for astronauts and on the mutation rate in microorganisms.

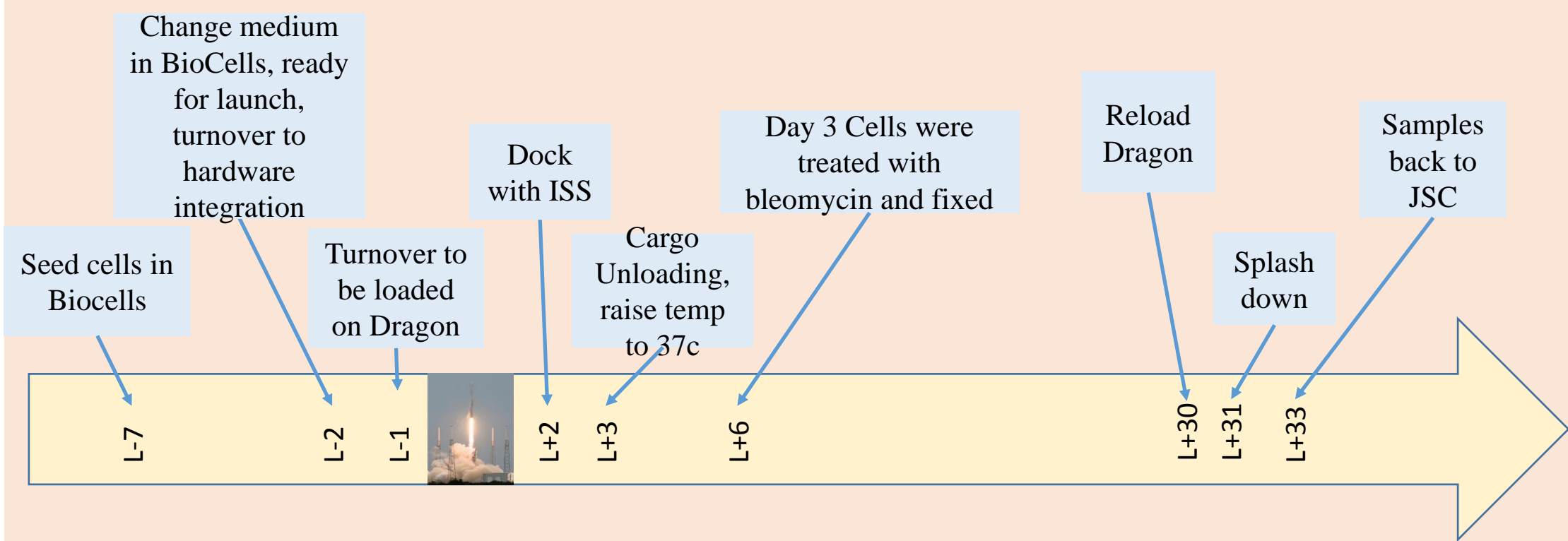
Although possible synergistic effects of space radiation and other spaceflight factors have been investigated since the early days of the human space program, the published results were mostly conflicting and inconsistent. To investigate effects of spaceflight on cellular responses to DNA damages, human fibroblast cells flown to the International Space Station (ISS) were treated with bleomycin for three hours in the true microgravity environment, which induced DNA damages including double-strand breaks (DSB) similar to the ionizing radiation. Damages in the DNA were measured by the phosphorylation of a histone protein H2AX ( $\gamma$ -H2AX), which showed slightly more foci in the cells on ISS than in the ground control. The expression of genes involved in DNA damage response was also analyzed using the PCR array. Although a number of the genes, including CDKN1A and PCNA, were significantly altered in the cells after bleomycin treatment, no significant difference in the expression profile of DNA damage response genes was found between the flight and ground samples. At the time of the bleomycin treatment, the cells on the ISS were found to be proliferating faster than the ground control as measured by the percentage of cells containing positive Ki-67 signals. Our results suggested that the difference in  $\gamma$ -H2AX focus counts between flight and ground was due to the faster growth rate of the cells in space, but spaceflight did not affect initial transcriptional responses of the DNA damage response genes to bleomycin treatment.

## Objectives

Investigate cellular responses to bleomycin-induced DNA damage in G1 human fibroblast cells in space.

## Experimental Design

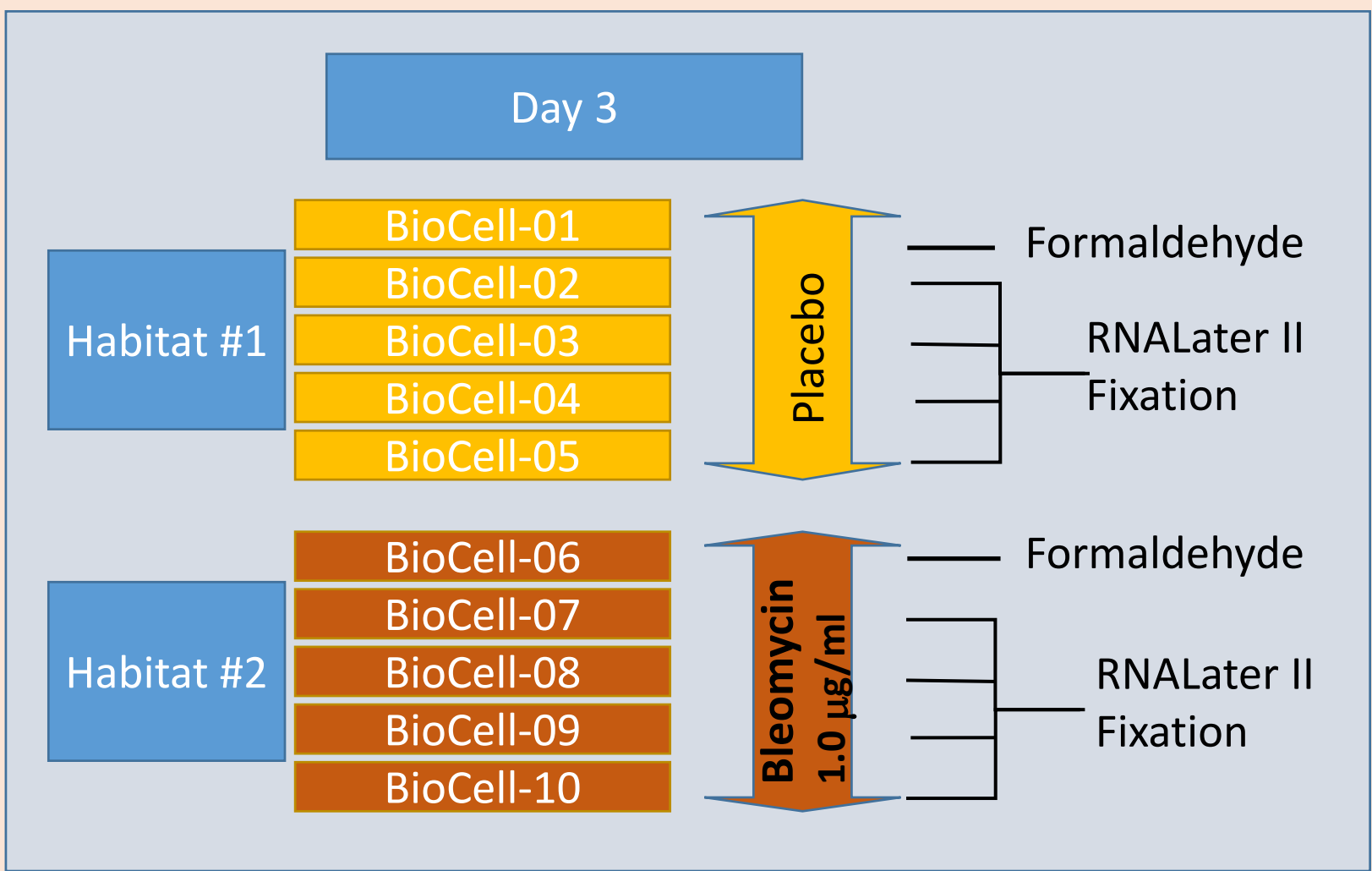
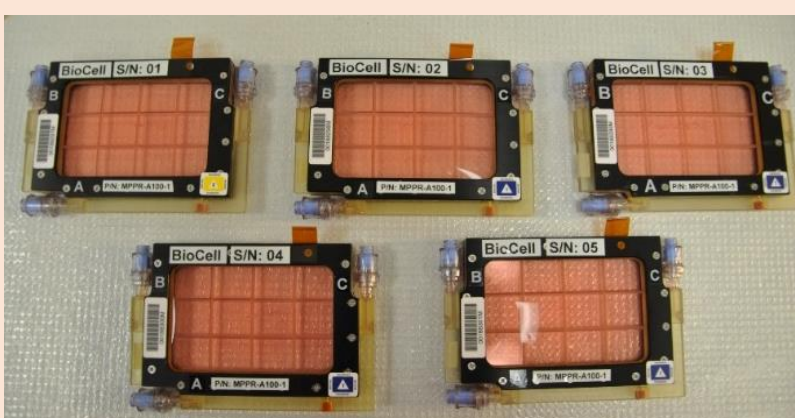
### Experimental Timeline



## Experimental Design (continued)

**Experimental Details:** Confluent human fibroblast AG1522 cells in BioCells were treated with placebo or 1.0  $\mu$ g/ml of Bleomycin for 3 hours, and then washed with PBS and fixed with formaldehyde or RNALater II. The cells were kept at 4C until returning to Johnson Space Centr (JSC). Ground experiments were performed exactly the same way as in space, but with a 6 hour offset from the flight schedule at Kennedy Space Center (KSC).

### BioCells

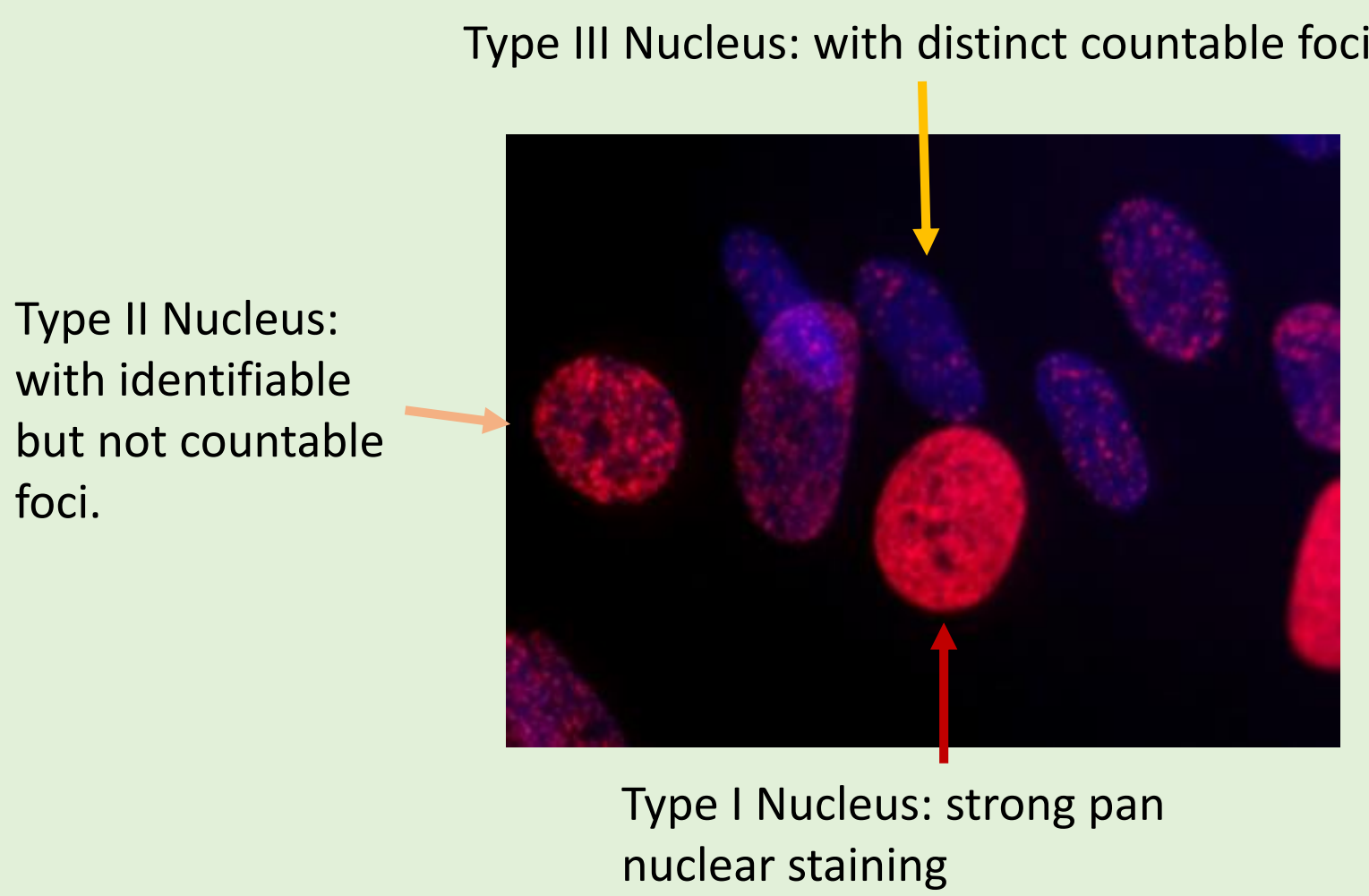


**Analysis:** Cells fixed with formaldehyde were subjected to immunofluorescence staining to access cellular proliferation (Ki-67) and the extent of DNA damages ( $\gamma$ H2Ax).

Total RNA isolated from RNALater II fixed cells was subjected to RT-PCR , using RT2 Profiler PCR Array Human DNA Damage Signaling Pathway (SA Biosciences, Qiagen) and Bio-Rad CFX96 Touch™ Real-Time PCR Detection System.

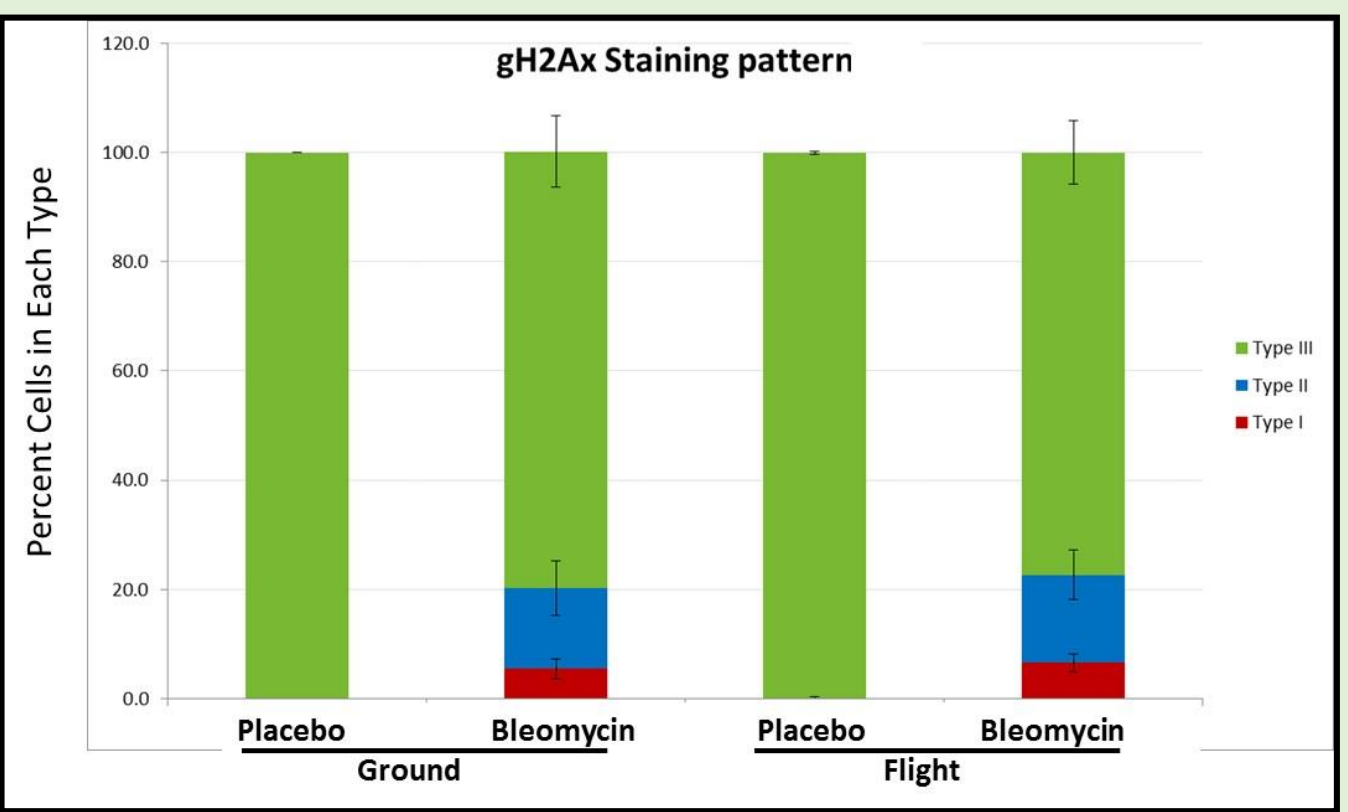
## Results

Examples of  $\gamma$ -H2AX staining patterns in confluent AG1522 fibroblast cells after three hours treatment of bleomycin.

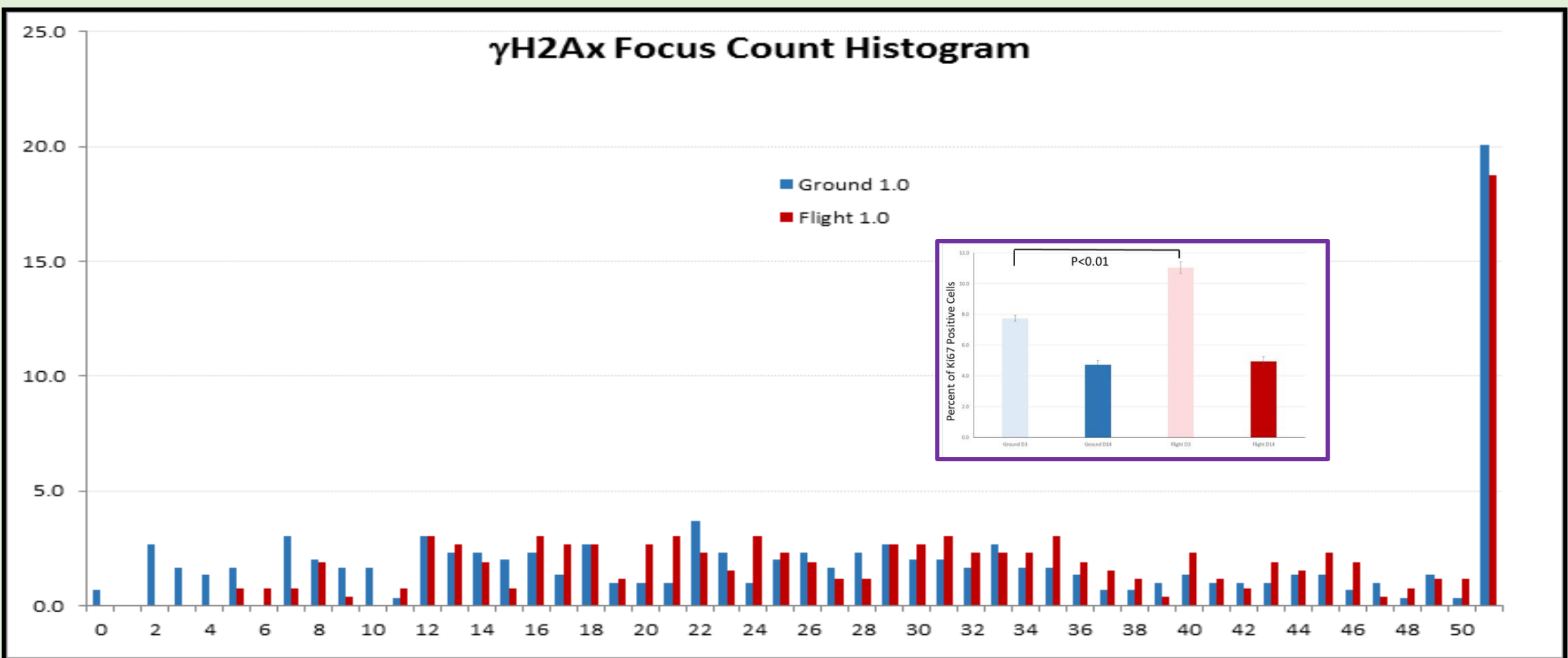


## Results (Continued)

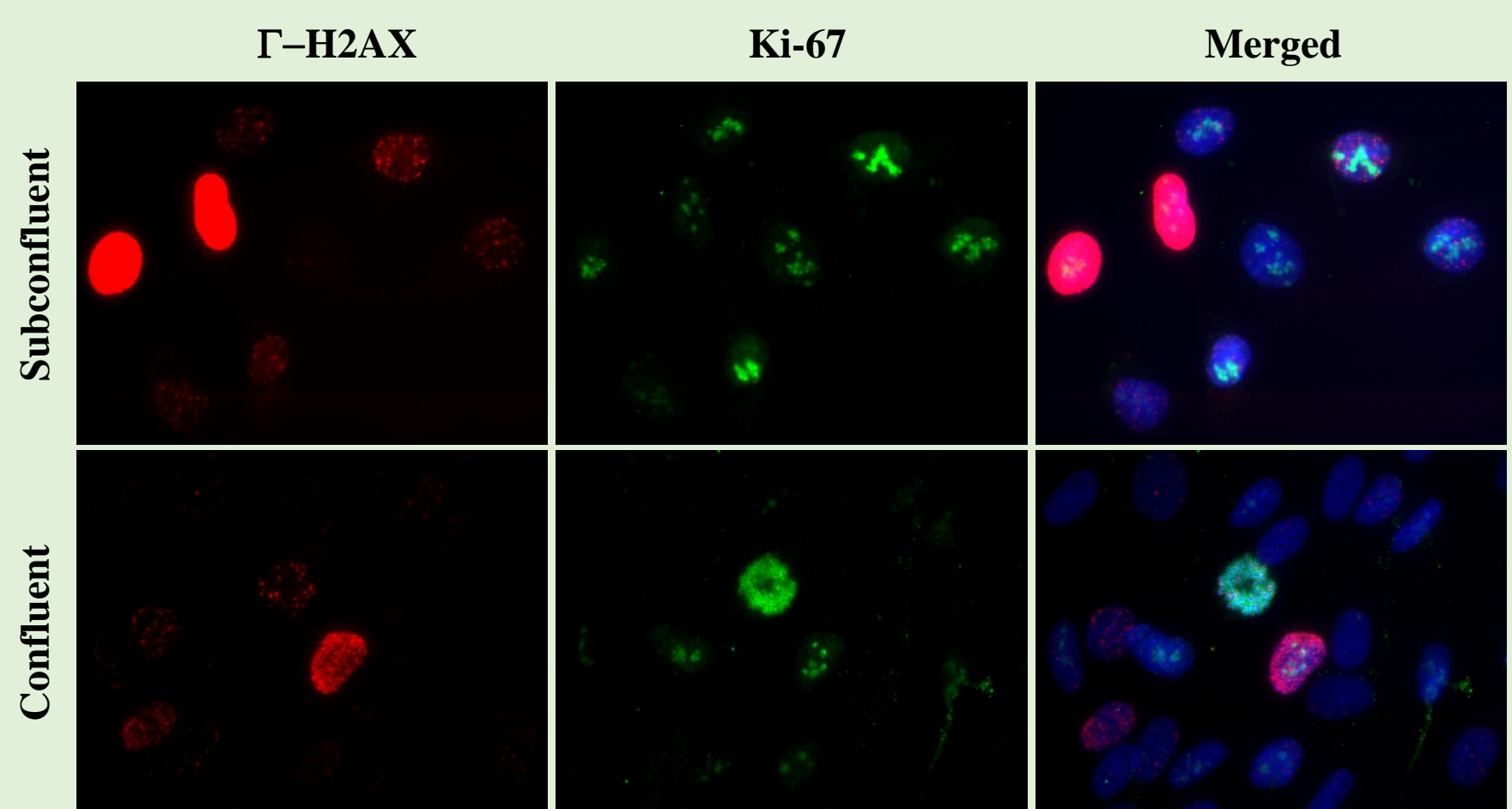
**Quantification of DNA damage by counting of  $\gamma$ H2Ax staining patterns (Type I, II, & III) showed no significant differences between the flown and ground cells.**



**Quantification of DNA damage by counting of  $\gamma$ H2Ax foci per nucleus (in Type III only) showed that cells in space flight were more sensitive to bleomycin treatment. Insert shows that there were slightly more proliferating cells in the flight population on Day 3.**



Control ground experiments confirmed that cells with higher proliferating population were more sensitive to bleomycin treatment. Shown below are examples of subconfluent cells (> 90% Ki-67 positive) were slightly more sensitive to bleomycin treatment of the same concentration.



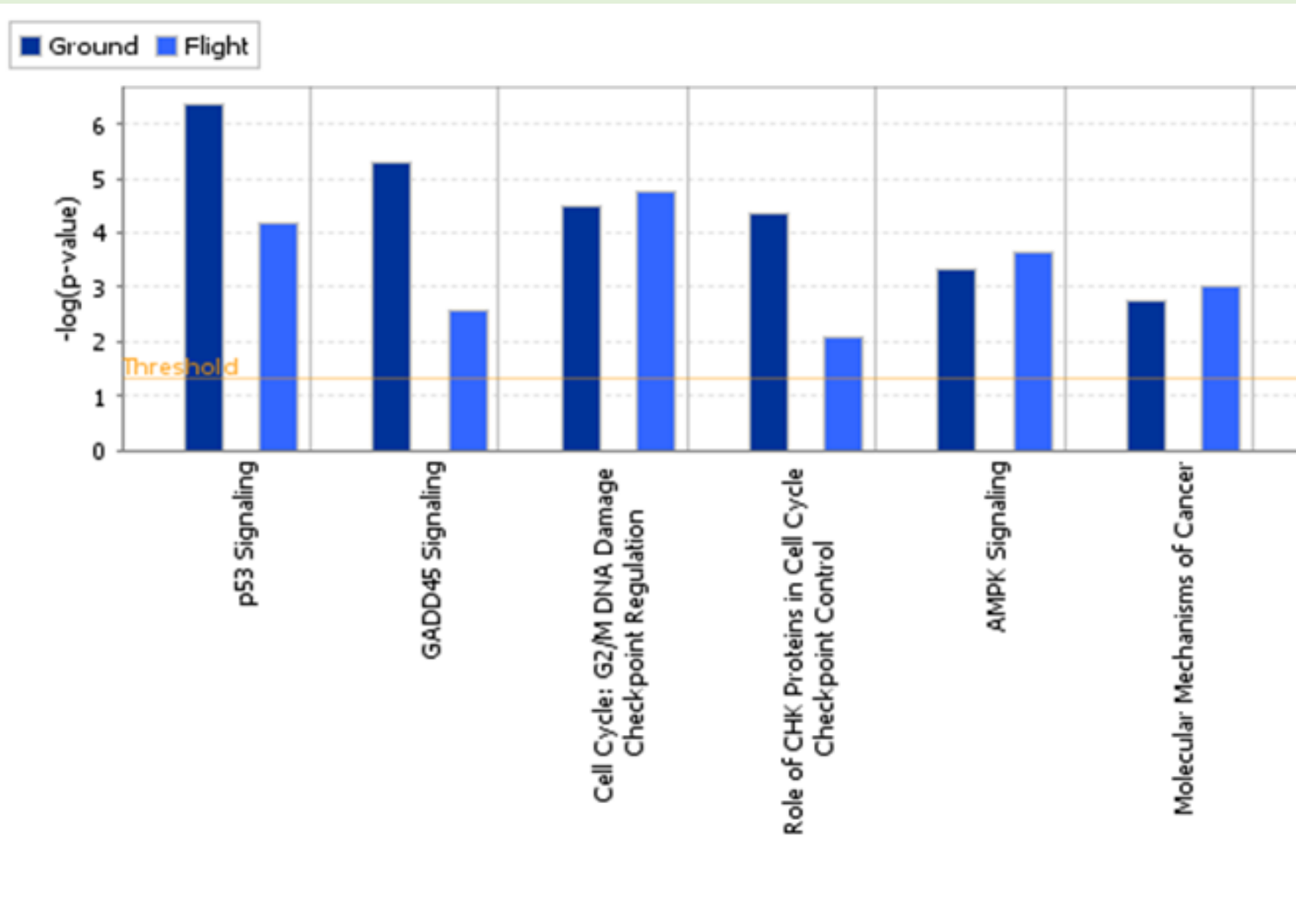
## PCRarray Study - DNA Damage Signaling

When comparing bleomycin treated to placebo treated samples, four genes whose expressions were significantly changed in both ground and flight samples after bleomycin treatment. This result is in agreement of our previous ground study results (manuscript in preparation).

Bleomycin treated vs placebo		
Genes	Ground	Flight
BBC3	↑	↑
CDKN1A	↑	↑
PPM1D	↑	↑
PCNA	↑	↑

Note: p < 0.05; Cutoff in fold change is set at 1.3

Top regulated canonical pathways for bleomycin treated samples in flight and ground.



## Conclusions

• Space flight had little effects on the cellular response to bleomycin-induced DNA damages in confluent human fibroblast cells as measured by the pattern of  $\gamma$ -H2AX.

• Spaceflight did not affect the response of the DNA damage response genes to bleomycin treatment in confluent human fibroblast cells.

• The slight difference in the  $\gamma$ -H2AX focus count between the flown and ground cells after bleomycin treatment was likely due to the faster growth rate of the cells in space.

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